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IL-6 Inhibited starvation-induced autophagy through STAT3/Bcl2/Beclin1 pathway

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Autophagy is widely existed as a lysosome-dependent degradative way in eukaryotic cells. Under normal conditions, autophagy is in the basal state, but in abnormal cases autophagy is then activated such as starvation, metabolic pressure, cyto-organelle damage and microbial infection. Some researches about the relationship between cytokines and autophagy have also received much more attention. IL-6, a pleiotropic cytokine secreted by many immune and immuno-related cells, can regulate cellular immunity and participate in the pathogenesis of some diseases. The main signal-transduction pathways downstream of IL-6/IL-6Rα/gp130 included JAK/STAT, PI3K/Akt and Ras/Erk pathway. These pathways also play an important role in the process of autophagy. Some investigators have also confirmed the effect of IL6 on autophagy. However these reports are controversial. Our results showed that the addition of IL-6 reduced the protein levels of LC3 and significantly activated the phosphorylation of STAT3 at Tyr705. Meantime the expression of Beclin1, a component of class III PI3K compex, was attenuated in U937 cells. Knockdown of Beclin1 by siRNA revealed its involvement in inhibition of autophagy by IL-6. STAT3 inhibitor LLL12 and the mutant STAT3Y705F suppressed the its phosphorylation and expression of Bcl-2 while enhanced expression of autophagy related proteins like LC3 and Beclin1. In conclusion, we proposed that p-STAT3 could mediate the inhibition of starvation-induced autophagy by exogenous IL-6. IL-6 could inhibit starvation-induced autophagy by STAT3/Bcl2/Beclin1 pathway in cells.

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IL10 inhibits induction of miR-155 by suppressing Ets2 – A possible mechanism for the anti-inflammatory effects of IL10

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MicroRNA-155 (miR-155) is highly expressed in many cancers such as B cell lymphomas and myeloid leukemia, and inflammatory disorders such as rheumatoid arthritis, atopic dermatitis and multiple sclerosis. The role of miR-155 as both a promoter of inflammation and an oncogenic agent provides a clear need for miR-155 itself to be stringently regulated. We have recently found that the immunomodulatory cytokine Interleukin 10 (IL-10) can potently inhibit miR-155 expression in response to stimulation of innate immune signaling pathways. We have found that IL-10 acts by suppressing the Ets family of transcription factors, which are required for miR-155 transcription. We found that IL-10 can act directly to control a number of Ets family members including Ets-2 and its repressor factor, ERF. ChIP assays, in addition to truncation and mutation studies, revealed a key Ets binding site through which IL-10 may act to inhibit miR-155. Inhibition of miR-155 by IL-10 acting via suppression of Ets2 is likely to be part of the molecular mechanism whereby IL-10 elicits its anti-inflammatory and immunomodulatory effects.

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Inducible nitric oxide synthase is upregulated by IL-23/IL-17A axis in inflammatory bowel disease: A study in Algerian patients

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Inflammatory bowel diseases (IBDs) are chronic inflammatory diseases of the gastrointestinal tract, which are clinically present as one of two disorders, Crohn's disease (CD) or ulcerative colitis (UC). The aim of our work was to study the involvement of Th17 subset in bowel disease pathogenesis by nitric oxide (NO) pathway in Algerian patients with IBD. We investigated the correlation between proinflammatory cytokines (IL17, IL23 and IL-6) and NO production in two groups of patients. We analyzed the expression of mRNAs encoding Th17 cytokines, cytokines receptors and NO synthase 2 (NOS2) in plasma of patients. In the same way, the expression of p-STAT3 and NOS2 was measured. We also studied NO modulation by proinflammatory cytokines (IL-17A, IL-6, TNF-α or IL-1β) in presence or absence of All Trans retinoic acid (At RA) in PBMC, monocytes and in colonic mucosa cultures. Analysis of cytokines, cytokines receptors and NOS2 transcripts revealed that levels of mRNA transcripts of the indicated genes are elevated in all IBD groups. Our study shows a significant positive correlation between NO and IL-17A, IL-23, IL-6 levels in plasma of IBD patients. Interestingly, the correlation is significantly higher in patients with active CD. Our study show that both p-STAT3 and iNOS expression were upregulated in PBMCs and colonic mucosa, especially in patients with active Crohn disease.

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The convergence of IL-1 and type I IFN on antiviral gene networks is regulated by distinct IRF family member cross-talk

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We have previously observed that IL-1β and type I IFN (IFNβ) cooperate to control viral replication within the CNS of infected animals and in viral target cells after challenge with West Nile virus (WNV). However, the mechanism by which these cytokine signaling pathways cooperate to elicit this immune control is not clearly defined. Using global transcriptome analysis, we have identified a distinct and targeted IL-1-driven "antiviral signature" comprised of known and novel host restriction factors involved in WNV control. Remarkably, co-treatment of myeloid cells with both IL-1β and IFNβ induced the expression of distinct subsets of this signature, revealing IL-1β-dependent, IFNβ-dependent, or synergistically driven gene induction profiles. Furthermore, within this signature we observed specific requirements for IRF3, IRF5, IRF7 and IFNAR activation, demonstrating that IL-1 driven host restriction factors may be regulated by distinct IRF interactions. Together these data demonstrate that inflammatory and antiviral signals integrate at the level of IL-1β and IFNβ to elicit IRF-specific targeted ISG expression and control of WNV. Furthermore, these data illuminate the possibility that specific host-driven viral signatures exist in a pathogen-dependent manner. Thus, strategies to co-opt these cytokine activated antiviral signatures may increase our ability to generate novel targeted therapeutic strategies tailored specifically to individual pathogens.

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Transcriptional signatures of pathogenesis and successful type I interferon and ribavirin therapy in a rhesus macaque model of MERS-CoV infection

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A novel betacoronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), emerged on the Arabian Peninsula in 2012. MERS-CoV was found to produce severe respiratory illness with case-fatality rates near 50%, and may be transmitted person-to-person in instances of close physical contact, such as among family members and to health care workers. As mice, hamsters, ferrets, and cynomolgus macaques are either not susceptible to MERS-CoV infection or do not develop observable clinical disease, we sought to determine whether rhesus macaques (*Macaca mulatta*) can be used as a model of MERS-CoV infection and disease. Animals challenged with MERS-CoV via multiple combined infection routes rapidly developed pneumonia, and we observed other clinical signs of illness, virus shedding, and virus replication in

respiratory tissues. Using microarrays to compare transcripts associated with histopathologic lesions removed from the lungs of infected animals, we identified 173 significant differentially expressed genes (DEG) associated with lung lesions on day 3 post-infection concurrent with peak clinical signs. Functional analysis indicated that these genes were predominantly associated with proinflammatory processes, recruitment and chemotaxis of inflammatory cells, and antiviral immunity. Using singular value decomposition coupled multidimensional scaling (SVD-MDS), we also identified changes in similar inflammatory genes in peripheral blood mononuclear cells, suggesting a rapid activation of innate immune and inflammatory processes in leukocytes after infection. These data were consistent with cytokine and chemokine profiles in the blood of animals.

Due to the urgent need for an effective therapeutic means of treating MERS-CoV patients, we used this rhesus macaque model to evaluate post-exposure treatment with combination interferon (IFN)-a2b and ribavirin (RBV), and identified key transcriptional signatures associated with disease severity and treatment efficacy. Animals treated with IFN-a2b/RBV beginning 8 h post-infection did not develop significant clinical or severe histopathologic signs of disease, breathing abnormalities, or radiographic evidence of pneumonia, and showed reduced viral loads and lower levels of serum proinflammatory cytokines. Microarray analysis of treated animals showed significant induction of interferon-stimulated genes (ISGs) and fewer upregulated transcripts associated with inflammatory processes. Additionally, we identified a subset of statistically significant DEGs associated with signaling pathways such as hedgehog, which has been linked to airway function and protection against pulmonary injury. Together, these data indicate that treatment of MERS-CoV-infected rhesus macaques with IFN-a2b and ribavirin reduces virus replication and improves clinical outcome, and suggests a transcriptomic basis for therapeutic efficacy. As these two drugs are already used together in the clinic, IFN-a2b and ribavirin may be useful for therapeutic intervention and patient management of new human MERS-CoV cases.

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IL-27 signals to both B and T cells support germinal center function and the development of GC-driven lupus

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IL-27 is a heterodimeric cytokine that shares sequence and structural homology with IL-12, IL-6 and IL-23. The bulk of the previous studies into IL-27 function have centered on its important role in directing CD4+T helper cell differentiation. The majority of *in vivo* models of inflammatory/autoimmune diseases and infection have indicated an immunosuppressive role for IL-27, through induction of IL-10 as well as direct suppression of TH17 and TH2 differentiation. For example, in models of T cell driven autoimmune disease, such as EAE, IL-27 suppresses the autoimmune response. However, our recent studies showed a surprisingly important pro-inflammatory role for IL-27 during germinal center responses. This effect was due, at least in part, to influences on T Follicular Helper (TFH) cell defects. We sought to investigate the effect of IL-27 signaling in a TFH driven model of lupus, the Sanroque mouse strain, by crossing IL27ra deficient mice with Sanroque mice. Deletion of IL27Ra reduced TFH and GC B cell numbers and ameliorated some aspects of disease in this model. Bone marrow chimeric approaches indicated that IL-27 signals directly to B cells contribute to development of disease. Although both chains of the IL-27 receptor are expressed by B cells, its effect on these cells during the antibody response is poorly characterized. We found that IL-27 stimulation of purified B cells *in vitro* enhances expression of the transcription factor Bcl-6 and promotes phenotypic features of GC B cells. Together, our data suggest that IL-27 signals to B cells play an important role in cell fate (i.e. GC versus plasma cell) determination and that these B cell specific effects contribute to the GC defect observed in IL-27Ra deficient mice.

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Understanding the mechanisms of interferon induction by a large DNA virus and its application to vaccine development

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African swine fever virus (ASFV) is a complex DNA virus and the aetiological agent of a domestic pig disease that can assume different clinical forms, ranging from an acute haemorrhagic disease to unapparent infections. The complexity of the immune response has impaired the development of an effective vaccine against ASFV infection. Previous work has established that immunization of pigs with attenuated replication competent ASFV strains can induce good levels of protection against lethal challenge with virulent strains. However in some pigs adverse clinical signs occur following immunization. On the other hand, due to the highly acute nature of the disease following infection with the virulent strains, it is clear that the porcine innate response is insufficient to prolong the life of the animal until the serological and cellular adaptive immune responses provide immediate protection and, importantly, the generation of the immunological memory crucial to vaccine development. The type I interferon (IFN) response is the first line of innate immunity against viral infection. Most, if not all viruses have to overcome this host defense before they can establish productive infection. We are currently characterizing the induction of type I IFN in primary porcine macrophages infected with strains of different virulence. A better understating of how the IFN response is modulated by these different viruses, will provide a rational basis for the development of novel vaccine strategies, for example, further attenuation of the virus by deleting genes involved in the evasion of the IFN host system is in progress.

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Towards the identification novel regulators for Th1 differentiation by computational genetics

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T helper (Th) cells play critical functions in response to infectious, allergic, and autoimmune diseases. Upon exposure to an infectious agent or stimulus, different types of Th responses are observed, which influence disease outcome, with differences in responses in different inbred mouse strains as well. To investigate the genetic factors that contribute to such differential immune responses, we have integrated computational genetic analysis and transcriptomic data to identify novel elements involved in Th1 differentiation. We determined the phenotypic profiles of in vitro differentiated Th1 cells from 16 inbred mouse strains by measuring the RNA and protein level of the Th1 signature cytokine (IFN©) at six time points. Then, according to the phenotypic ranking of 16 inbred strains, we performed a haplotype-based computational genetic analysis. At all time points, we observed strong and significant interstrain differences, which suggested that the observed variations were due to genetic differences, and we identified ~1075 genes ($p < 0.001$) with polymorphisms that potentially contribute to the quantitative difference in IFN© expression. Many well known Th1 associated genes were found in this pool, including IFN© receptor, JAK2, STAT1, STAT4 and IRF4. We also established the transcriptomic profiles of Th1 cells from 4 mice strains by RNAseq analysis. As compared to naive $CD4^+$ T cells, ~80 genes had a significantly change in expression (FC > 2, $P < 1e{-}10$) in differentiated Th1 cells and had similar expression patterns to that of IFN© in the 4 mice strains, whereas ~50 genes showed a pattern opposite to that of IFN©. After combining these results with SNP data, we are now evaluating candidate genes for novel roles in regulating IFNγ expression upon Th1 differentiation.

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Characterization of human interferon subtype and interferon-stimulated gene signatures

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Type I interferons (IFNs) are expressed by all nucleated cells in response to viral pathogens and modulate innate and adaptive immune responses by stimulating expression of subsets of interferon stimulated genes (ISGs). There are over 1000 ISGs, including more than 60 that are transcription factors (TFs). Type I IFNs share the same receptor complex and are comprised of multiple species, including IFN-beta, IFN-omega, and twelve subtypes of IFN-alpha. Both type I IFNs and the more recently described type III IFNs activate STAT1/STAT2 heterodimers and stimulate additional pathways that translate into antiviral and antiproliferative responses. It is not well understood how the timing and dose of each IFN contributes to these functional outcomes. We hypothesize that expression patterns of TFs will reveal unique functional